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ON-LINE METABOLIC MONITORING OF TISSUE ISCHEMIA

BY

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To study the ability of extravascular myocardial tissue pH (MpHe) measured with an intramural electrode to reflect myocardial intracellular metabolic and myocardial function status during ischemia, 14 open-chest dogs had in vivo myocardial ^{31}P NMR spectroscopy during left anterior descending coronary artery (LAD) occlusion (experimental group, ischemic n = 7) or following sham operation (control group, non-ischemic n = 7). Spectra were acquired q5min at 4.7 tesla (256 averages, TR=1000secc, pulse width=30 us) with a 2 cm 2-turn RF surface coil. Regional myocardial function was assessed as percent systolic

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shortening (%SS) with simultaneous on-line sonomicrometer crystals in the ischemic LAD and a non-ischemic (left circumflex coronary artery LCX) zone. Left ventricular (LV) and diastolic pressure (LVEDP) and rate of pressure development (dp/dt) were assessed with a high-fidelity micromanometer in the LV. Intracellular myocardial phosphocreatine (PCr), adenosine triphosphate (ATP), and inorganic phosphate (PI) peak areas were normalized to an external phosphate standard (hexchlorocyclotriphosphazene, HCCTP). Changes in peak areas and %SS are expressed as percent baseline values. After 60 minutes of ischemia, MpHe fell from 7.38 $\pm$0.02 to 5.96$\pm$0.09, NMR calculated myocardial pH (mpHi) fell from 7.19 $\pm$ 0.02 to 6.07 $\pm$ 0.08, LAD %SS fell from 100% to 9% $\pm$ 16% control value. PCr fell to 21 $\pm$ 3% control, and ATP fell to 45 $\pm$ 3% control, (all $p<0.0001$). Pi rose to 1144 $\pm$ 166% control ($p=0.0007$). LCX %SS fell to 83 $\pm$ 6% control ($p=0.04$) in the ischemic group. LVEDP, and LV dp/dt did not change significantly during the ischemic interval. All parameters did not change significantly in the control animals. In the ischemic group, MpHe correlated with MpHi in each dog with an average r value of 0.96, $p<0.0001$: the fall in MpHi correlated with the loss of ATP in each dog with an average r value of 0.94, $p<0.0001$: and the fall in MpHe correlated with the loss of ATP in each dog with an average r value of 0.94, $p<0.0003$. Thus, myocardial pH, easily measured with an intramural electrode, correlates with NMR-derived myocardial pH and loss of myocyte ATP content, and reflects myocardial dysfunction during ischemia.

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ABSTRACT: To study the ability of extravascular myocardial tissue pH (MpHe) measured with an intramural electrode to reflect myocardial intracellular metabolic and myocardial functional status during ischemia, 14 open-chest dogs had *in vivo* myocardial ^{31}P NMR spectroscopy during left anterior descending coronary artery (LAD) occlusion (experimental group, ischemic n=7) or following sham operation (control group, non-ischemic, n=7). Spectra were acquired q5min at 4.7 Tesla (256 averages, TR=1000msecc, pulse width=30 us) with a 2 cm 2-turn RF surface coil. Regional myocardial function was assessed as percent systolic shortening (%SS) with simultaneous on-line sonomicrometer crystals in the ischemic LAD and a non-ischemic (left circumflex coronary artery LCX) zone. Left ventricular (LV) end diastolic pressure (LVEDP) and rate of pressure development (dp/dt) were assessed with a high-fidelity micromanometer in the LV. Intracellular myocardial phosphocreatine (PCr), adenosine triphosphate (ATP), and inorganic phosphate (PI) peak areas were normalized to an external phosphate standard (hexchlorocyclotriphosphazene, HCCTP). Changes in peak areas and %SS are expressed as percent baseline values. After 60 minutes of ischemia, MpHe fell from 7.38 $\pm$ 0.02 to 5.96 $\pm$0.09, NMR calculated myocardial pH (mpHi) fell from 7.19 $\pm$0.02 to 6.07 $\pm$0.08, LAD %SS fell from 100% to 9% $\pm$ 16%

control value. PCr fell to 21 ±3% control, and ATP fell to 45 ±3% control, (all p<0.0001). Pi rose to 1144 ± 166% control (p=0.0007). LCX %SS fell to 83 ±6% control (p=0.04) in the ischemic group. LVEDP, and LV dp/dt did not change significantly during the ischemic interval. All parameters did not change significantly in the control animals.
In the ischemic group, MpHe correlated with MpHi in each dog with an average r value of 0.96, p <0.0001: the fall in MpHi correlated with the loss of ATP in each dog with an average r value of 0.94, p <0.0001: and the fall in MpHe correlated with the loss of ATP in each dog with average r value of 0.94, p <0.0003. Thus, myocardial pH, easily measured with an intramural electrode, correlates with NMR-derived myocardial pH and loss of myocyte ATP content, and reflects myocardial dysfunction during ischemia.

INTRODUCTION: The aim of this research proposal is to elucidate the simultaneous changes in myocardial pH determined using nuclear magnetic resonance spectroscopy (NMR pH) and a second, independent measurement of extravascular myocardial pH during normothermic ischemia and reperfusion, and to correlate observed pH changes with changes in myocardial bioenergetic profile and myocardial function. In addition, the effects of hypothermia on myocardial function, and fibrillation threshold during ischemia are to be assessed. This report details the

progress of development of the normothermic ischemic canine model.

Extravascular myocardial pH is measured with a myocardial intramural electrode system developed in Dr Khuri's laboratory that has been shown to accurately and reliably reflect the degree of myocardial ischemia and resultant cellular ultrastructural damage. Myocardial NMR pH is determine by 31Phosphorus NMR spectroscopy utilizing a surface coil radio frequency transmitter/receiver developed at the Magnetic Resonance Imaging Facility at the Massachusetts Institute of Technology.

METHODS: Twenty male dogs were premedicated with acepromazine (0.10 mg/kg SQ), and anesthesia was induced with sodium pentobarbital (30 mg/kg IV), and maintained with supplemental doses hourly (1-5 mg/kg IV), as needed. The trachea was intubated with a cuffed endotracheal tube and the animal was ventilated with a volume ventilator and supplemental oxygen. Arterial blood gases were monitored with a blood gas analyzer, and minute ventilation was adjusted to maintain normal arterial gas tensions.

The heart was exposed through a left thoracotomy through the fifth intercostal space, and was suspended in a pericardial cradle. A large bore catheter was placed in the external jugular vein for the administration of fluids and medications. A fluid-filled catheter was placed in a carotid artery and connected to a Statham p23 ID transducer

via a three-way stopcock for measurement of arterial pressure and removal of blood gas specimens. The left anterior descending (LAD) coronary artery was dissected free between the first and second diagonal branches and an inflatable pneumatic balloon occluder was placed around it. The LAD was transiently occluded to define an area of epicardial cyanosis, the center of which was the area in the LAD distribution used in further instrumentation. A pair of 5 MHz piezoelectric ultrasonic crystals (constructed in our laboratory) were placed at the subendocardial level in the LAD distribution to measure myocardial segmental systolic shortening as an assessment of regional contractility. A second pair of crystals were placed in the distribution of the left circumflex coronary artery (LCX) to serve as a control. A Koningsberg Instrument (Pasadena, CA) high-fidelity micromanometer was then placed in the left ventricle via an apical stab wound to allow continuous measurement of left ventricular end-diastolic pressure (LVEDP), developed left ventricular pressure (LVP), and its first time derivative (dp/dt). The latter parameter was used to define the phases of the cardiac cycle (end systole and end diastole) for analysis of the ultrasonic segment length tracings (end diastolic length, EDL: and end systolic length, ESL). Percent systolic shortening (%SS) was calculate from the formula: $\%SS = \{(EDL-ESL)/EDL\} \times 100$. A glass pH electrode was inserted 10 mm into the myocardium in the region of the LAD and was connected to a voltmeter. A

reference electrode placed in 3M KCl solution was connected to the animal's front limb using a 3M KCl agar salt bridge. Anterior myocardial wall temperature was measured using a fiberoptic phosphorus temperature probe(Luxtron Fluoroptic Temperature Sensing, Mountain View. CA). A two turn NMR surface coil with an internal diameter of 2.0 cm was placed over the area where the pH electrode was located and sewn to the epicardium so that the sensing portion of the pH electrode was in the center of the surface coil. A solution of 1M hexachlorocyclotriphosphazene (HCCTP) and 0.062M chromium acetylacetonate in benzene was placed on a sealed 35 ul flask and affixed to the surface coil to serve as an external phosphate standard for the determination of relative phosphate metabolic peak areas on each NMR spectrum. The open chest was then wrapped in a warming blanket to maintain myocardial temperature. Hemodyanmic data including arterial and left ventricular pressures, the derivative of left ventricular pressure, and ultrasonic segment length tracings were recorded continuously on an eight channel Gould chart recorder. Hemodynamic data were recorded at chart recorder at a chart speed of 100mm/sec at the end of the expiratory cycle. Myocardial pH and temperature data were recorded continuously on a Soltec strip chart recorder.

Once instrumentation was complete, the surface coil was tuned to the resonant frequency of 81.0001 MHz for 31Phosphorus NMR spectral acquisition at 4.7 Tesla. The

animal was then introduced into a 35 mm bore Oxford Instruments magnet with a field strength of 4.7 Tesla. After shimming the magnetic field to optimize field homogeneity based on the proton resonant frequency of 200.1 MHz at 4.7 Tesla, ^{31}P spectral acquisition was performed. Fully relaxed spectra were obtain by averaging 128 signal acquisitions, each with a pulse width of approximately 45 microseconds and a relaxation delay of 20 seconds, over a period of 45 minutes. Partially saturated spectra were obtained by averaging 256 signal acquisitions, each with a pulse width of approximately 30 microseconds and a relaxation delay of 1 second, over a period of 5 minutes. We have found these parameters of spectral acquisitions to give an excellent signal to noise ratio that allows for quantitative assessment of both NMR-derived myocardial pH and high energy phosphate peak areas during in vivo surface coil spectroscopy in the dog. The free induction decay information for each spectrum was stored in a data file using an IBM CS 9000 computer and IBM MYSTIC software for off-line data analysis. Each NMR spectrum was analyzed by the same observer (I.K.) utilizing the commercially available NMR1 software package (New Methods Research Inc) on a Sun 3 computer system. The free induction decay information was processed by applying exponential multiplication with 15 Hz line broadening prior to Fourier Transformation. After transformation, each spectrum was phased with baseline deconvolution, to produce a spectrum

relating peak intensity to frequency (or chemical shift, parts per million, ppm). Myocardial NMR pH was calculated from the unmodified spectrum based on the chemical shift of the inorganic phosphate peak from the phosphocreatine peak in ppm using the formula:

$$pH = 6.77 + \log \{ (3.29 - ppm) / (ppm - 5.68) \}$$

High energy phosphate compound spectral peak areas including inorganic phosphate (PI), phosphocreatine (PCr), and adenosine triphosphate (ATP), were quantitated after subjecting each raw spectrum to a Lorentzian curve fitting routine. The resulting spectrum then had assignment of relative peak areas based on the phosphate standard (HCCTP) peak being assigned to an area of 100%. Only the beta peak of ATP was used for ATP peak analysis, as both the alpha and gamma peak areas contain contributions from other phosphorylated nucleotides and bases. Peak area data thus derived was expressed as a percentage of pre-ischemic control levels. Partially saturated spectra collected under identical conditions were used for both the pre-ischemic control and ischemic spectral peak area determinations and no correction was applied for the degree of partial saturation as only relative peak areas and relative changes in peak area were used in data analysis.

EXPERIMENTAL PROTOCOL: In all animals, after instrumentation and placement in the magnet, a 60 minute stabilization period was allowed during which a fully

relaxed spectrum was obtained. Following this, four identical partially saturated, pre-ischemic spectra were obtained along with baseline hemodynamic, arterial blood gas, myocardial pH and temperature, and systolic shortening data.

After baseline data acquisition, the left anterior descending coronary artery was completely occluded by inflation of the balloon occluder. Repeated measurements of hemodynamic data were obtained to coincide with NMR spectral acquisition every five minutes for a period of sixty minutes. Animals were maintained on an intravenous lidocaine arip (2 mg/min) throughout this protocol, and were bolused with 50 mg of lidocaine just prior to the onset of ischemia to reduce the incidence of lethal ventricular fibrillation. A total of twenty dogs were instrumented as described. Seven animals survived after initiation of ischemia and served as the <u>experimental</u> group. Seven animals were identically instrumented, but did not have the LAD occluder inflated, and thus served as a non-ischemic <u>control</u> group. Six animals died from ventricular fibrillation, all within the first ten minutes of ischemia, and were excluded from the data analysis.

DATA ANALYSIS: Hemodynamic parameters are expressed as mean values $\pm$ the standard error of the mean (SEM). Percent systolic shortening is expressed as the mean percent control of the baseline value $\pm$ SEM. Extravascular myocardial pH data was calculated as the five minute time-coincident

average pH value during spectral acquisition from the continuous extravascular pH tracing. Pi, PCr, and ATP peak areas were expressed as the mean percent control of baseline non-ischemic relative peak areas ± SEM

Data analysis was performed using multivariate repeated measures analysis of variance (MANOVA) to detect significant differences in parameter values between the ischemic group and the non-ischemic group as well as significant changes within a group over time. When the analysis of variance indicated a significant difference with respect to time, the paired t-test was used to make specific timepoint comparisons within a group. Significance was determined at the $p < 0.05$ level. Simple linear regression was used to perform correlation analysis within each dog individually, as well as on the means of indicated parameters within the ischemic group. Significance was determine at the $p < 0.05$ level, and the Pearson correlation coefficient, r, is presented.

RESULTS: The mean myocardial temperature in both groups throughout the experiment was 36 C. Baseline hemodynamic and pH values for the control and experimental groups are shown in Table 1. Although there was a statistically significant difference between the control and experimental groups for LVEP and +dp/dt max at baseline, these differences are not considered to be important physiologically, and the two groups are considered

comparable. There were no significant changes in mean arterial pressure (MAP) left ventricular pressure, end diastolic pressure (LVEDP), and maximum rate of left ventricular pressure development (+ dp/dt max) in either the control or experimental group during the period during the periods of ischemia. There were no significant difference in heart rate between the two groups during the study interval.

An example of an unmodified (i.e., non-curvefit) baseline spectrum is shown in Figure 1. The excellent signal to noise ratio is clearly demonstrated and the peak areas resulting from the phosphate standard (HCCTP), inorganic phosphate (Pi), phosphocreatine (PCr), and the alpha, beta, and gamma peaks of adenosine triphosphate (ATP) are readily discernible. Figure 2 shows a stack plot of seven sequential spectra taken every five minutes during the first thirty minutes of ischemia in one animal. It is easy to appreciate the rapid increase in the Pi peak area, the rapid decrease of the PCr peak area, and the gradual fall in beta-ATP peak area as ischemia progresses. These spectra are representative of the observations made in the ischemic experimental group.

The extravascular myocardial pH was significantly higher than the NMR myocardial pH at baseline in both the control and experimental groups ($p<0.02$), and this pH gradient remained significantly different in the control group throughout the study interval ($p<0.05$). In the

experimental group the extravascular myocardial pH fell during ischemia from 7.38 ± 0.02 to 5.96 ± 0.09 after 60 minutes, which was significantly different from the change in the control group (7.35 ± 0.04 to 7.33 ± 0.04, $p < 0.03$). Similarly, myocardial NMR pH fell from 7.19 ±0.02 to 6.07 ±0.08 in the ischemic, experimental group ($p = 0.0001$) as apposed to the control group whose NMR pH did not change (7.20 ± 0.01 to 7.19 ± 0.01) during this interval. The changes in extravascular and NMR pH in the control and experimental groups are displayed graphically for the first thirty minutes of ischemia in Figure 3.

The loss of regional myocardial function with the onset of ischemia was rapid, occurring within the first minute of ischemia. In the experimental group, the left anterior descending (LAD) percent control systolic shortening (%CSS) fell from 100% to 9 ± 16% control value after sixty minutes of ischemia ($p = 0.001$) while there was no change in the LAD %CSS in the control group. This is demonstrated graphically for the first thirty minutes of ischemia in Figure 4. In the left circumflex distribution (LCS), the %CSS fell from 100% to 83 ± 6% control value ($p = 0.04$) in the experimental group after sixty minutes but did not change significantly in the control group.

With the onset of ischemia, the PCr level fell from 100% to 31 ±2% control value after five minutes and fell to 21 ± 3% at sixty minutes (both $p = 0.0001$). PCr did not change significantly in the control group. ATP loss

occurred in a more gradual fashion, going from 100% to 91 ± 2% control value after five minutes ($p = 0.0001$), and to 45 ± 3% control value after sixty minutes ($p = 0.0001$). ATP did not change significantly in the control group. These metabolite changes are depicted graphically for the first thirty minutes of ischemia in Figure 5. By contrast, Pi rose from 100% to 531 ±43% after five minutes, and to 1144 ± 166% after 60 minutes in the experimental group (both $p < 0.0007$), but did not change significantly in the control group.

To relate observed changes in extravascular myocardial pH to observed changes in NMR myocardial pH, linear regression was performed over the sixty minute ischemic period in each experimental animal. The results of this analysis are shown in Table II. Extravascular pH correlated with NMR pH with an average r value of 0.96 for the seven dogs, $p < 0.0001$. To relate observed changes in extravascular myocardial pH to the change in myocyte ATP content during ischemia, linear regression was performed over the same time interval in each experimental animal. The results are shown in Table III. Extravascular pH correlated with observed percent control ATP content with an average r value of 0.94 for the seven dogs, $p \leq 0.0003$). A similar analysis between NMR myocardial pH and percent control ATP content demonstrated an average r value of 0.94 for the seven dogs, $p \leq 0.0001$.

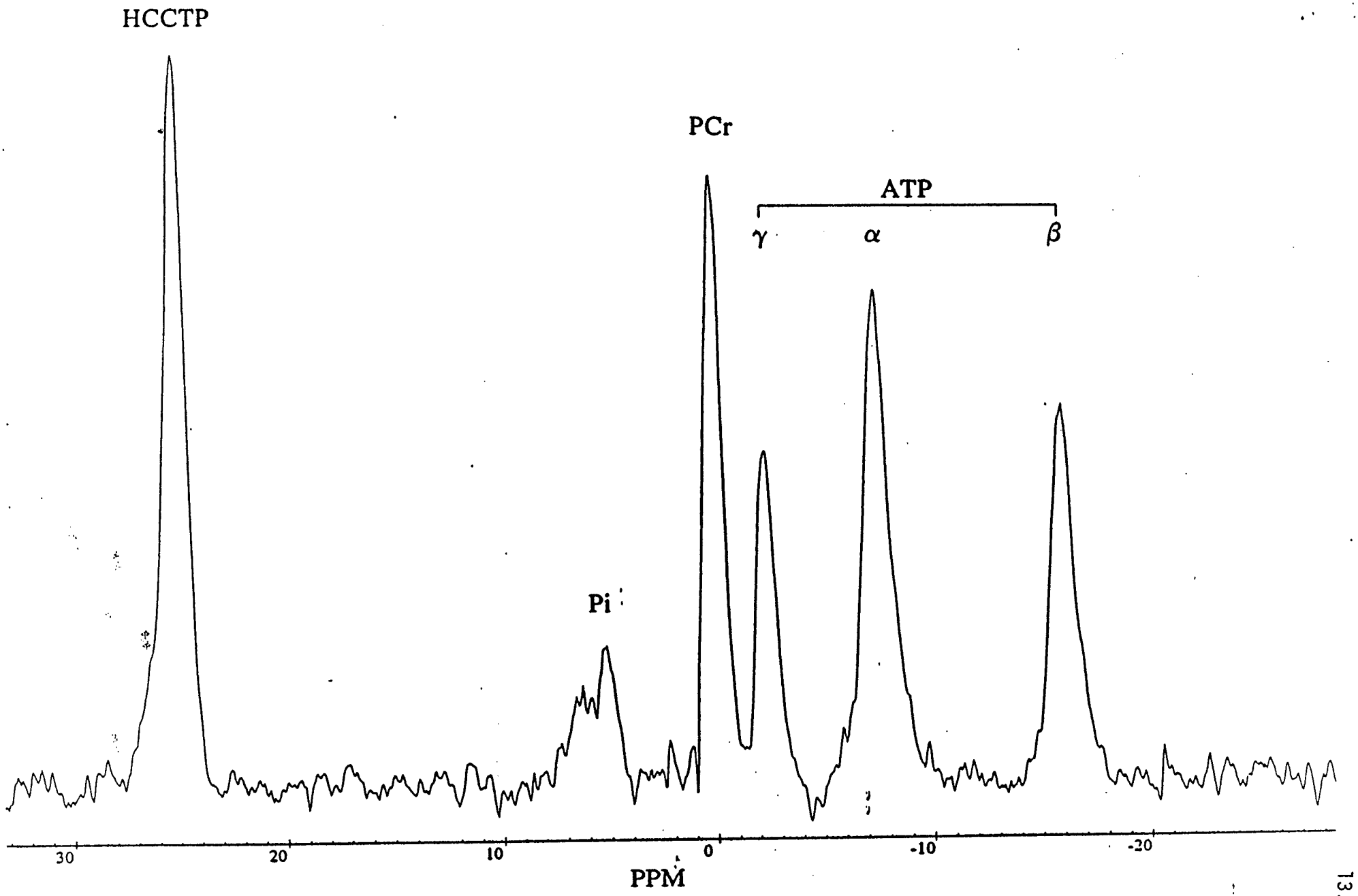

FIGURE 1: MYOCARDIAL 31 PHOSPHORUS NMR SPECTRUM IN VIVO

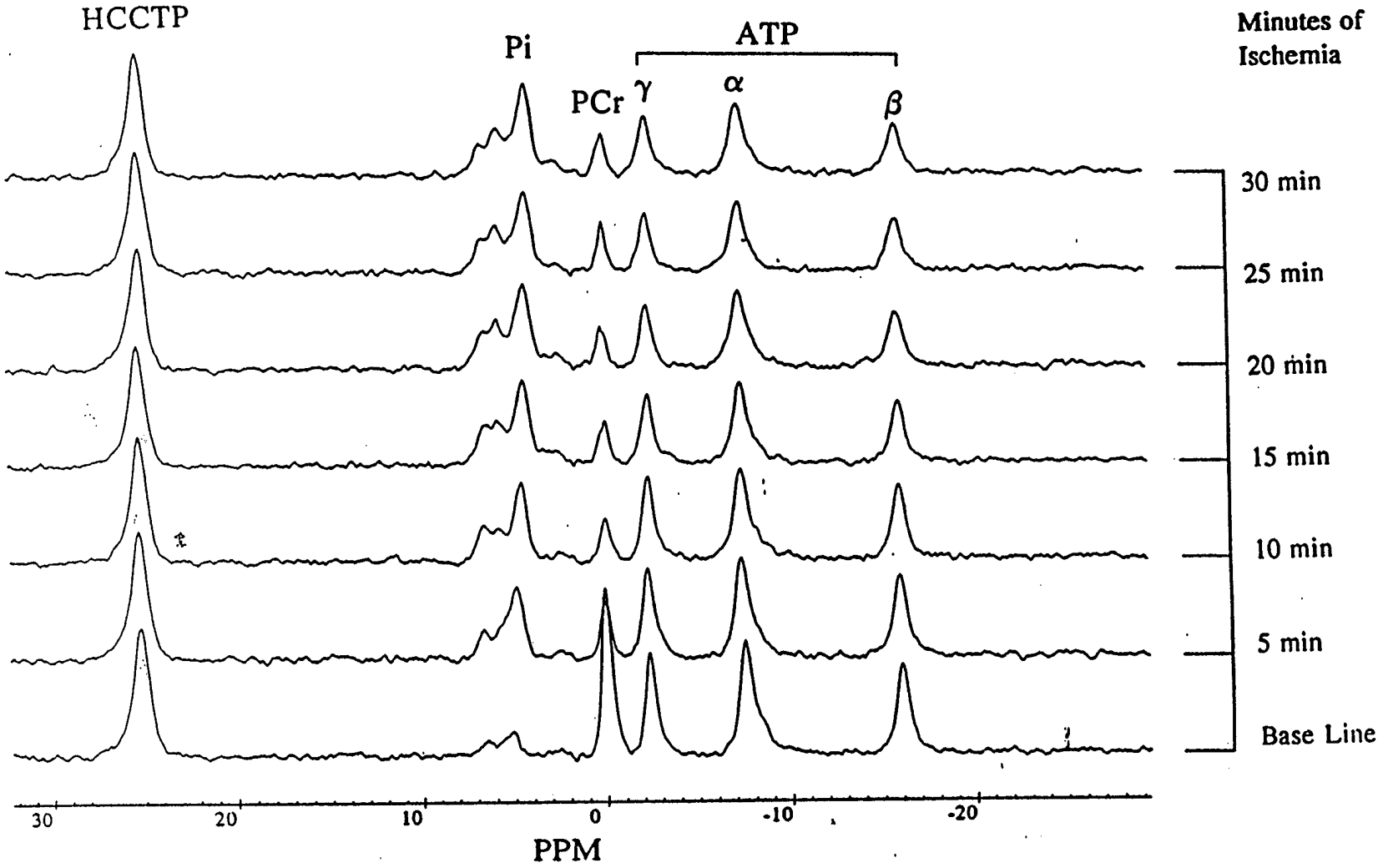

FIGURE 2: MYOCARDIAL 31 PHOSPHORUS NMR SPECTRA DURING ISCHEMIA

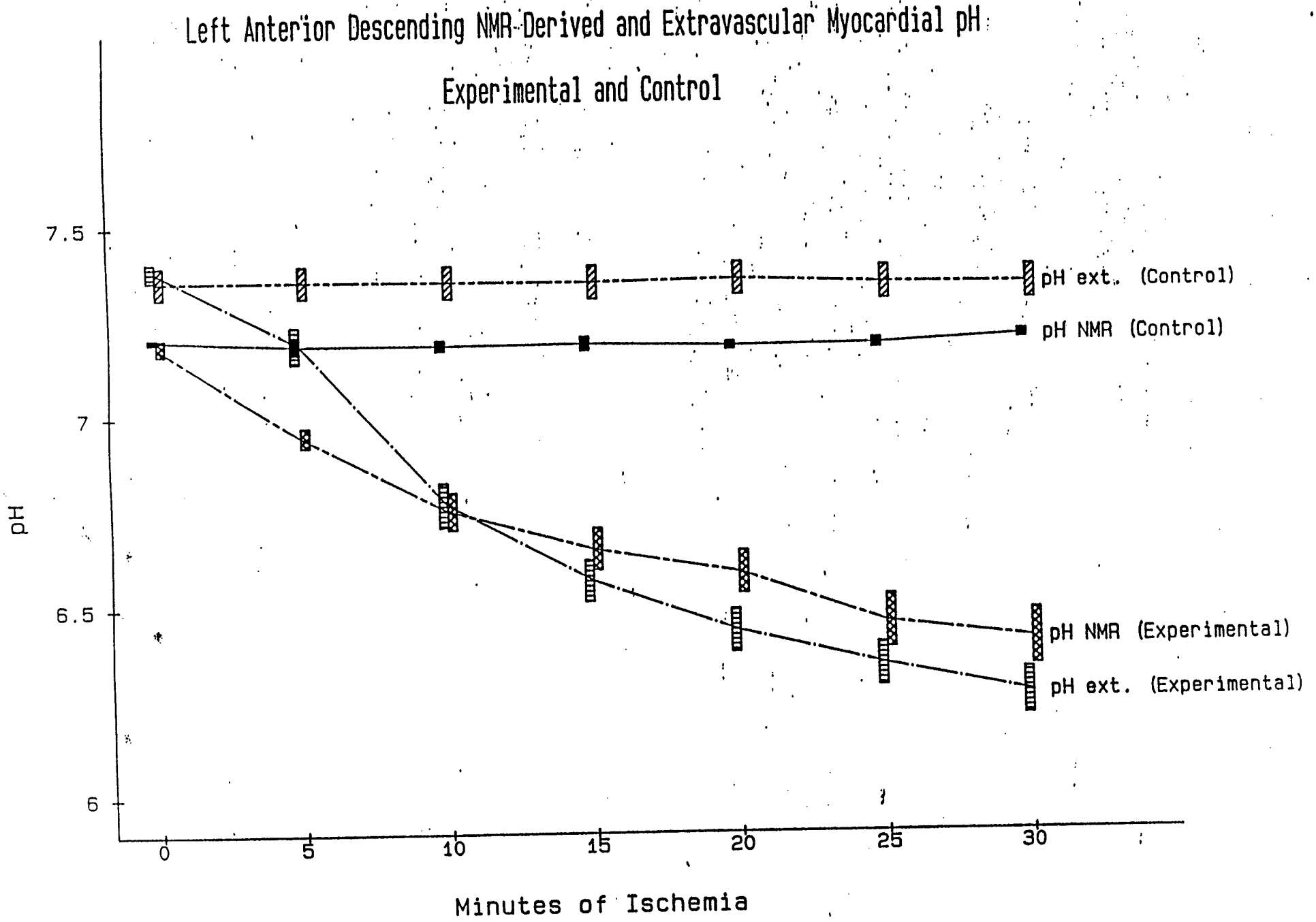

FIGURE 3: MYOCARDIAL PH CHANGES DURING THIRTY MINUTES OF ISCHEMIA

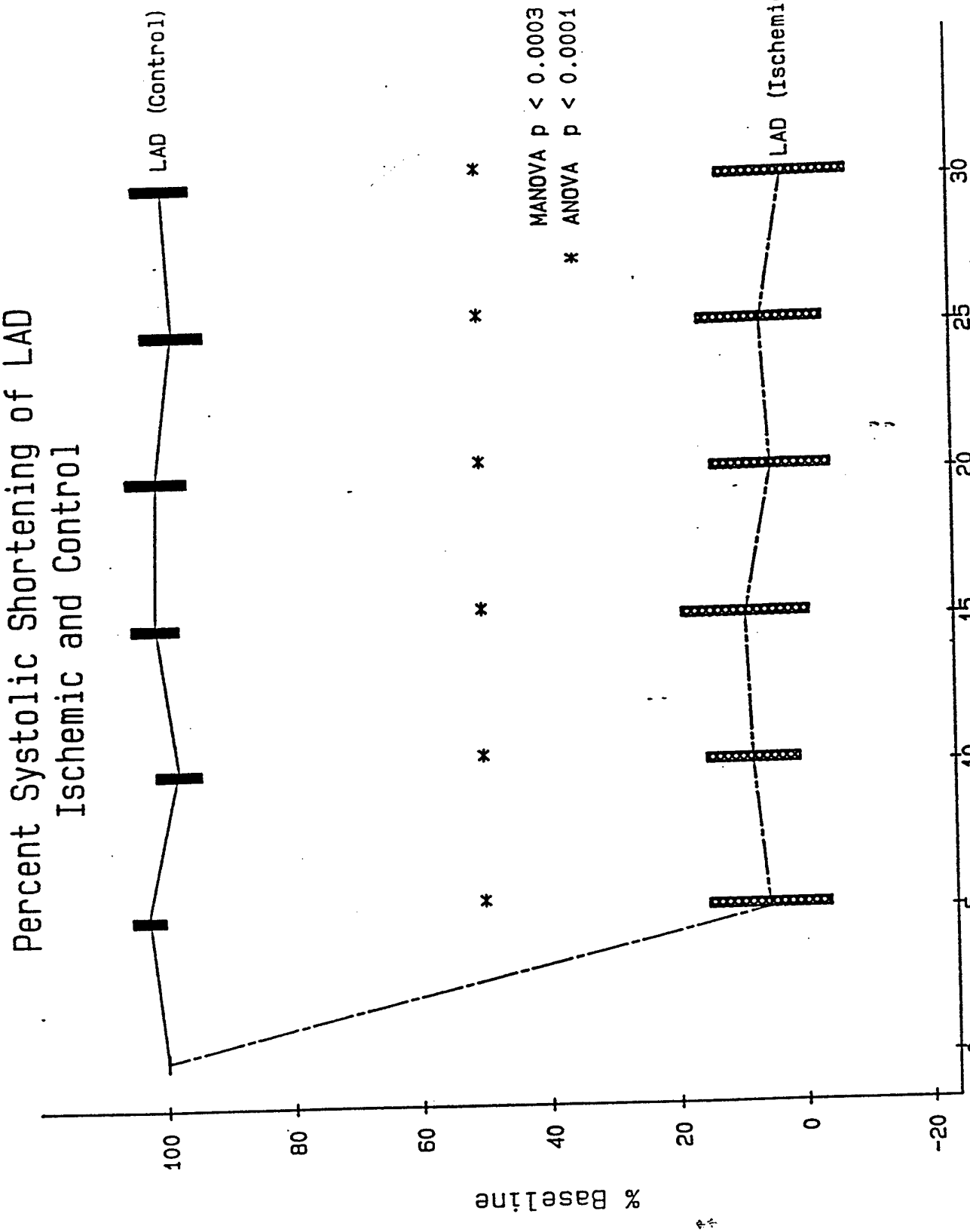

FIGURE 4: MYOCARDIAL REGIONAL FUNCTIONAL CHANGES DURING THIRTY MINUTES OF ISCHEMIA

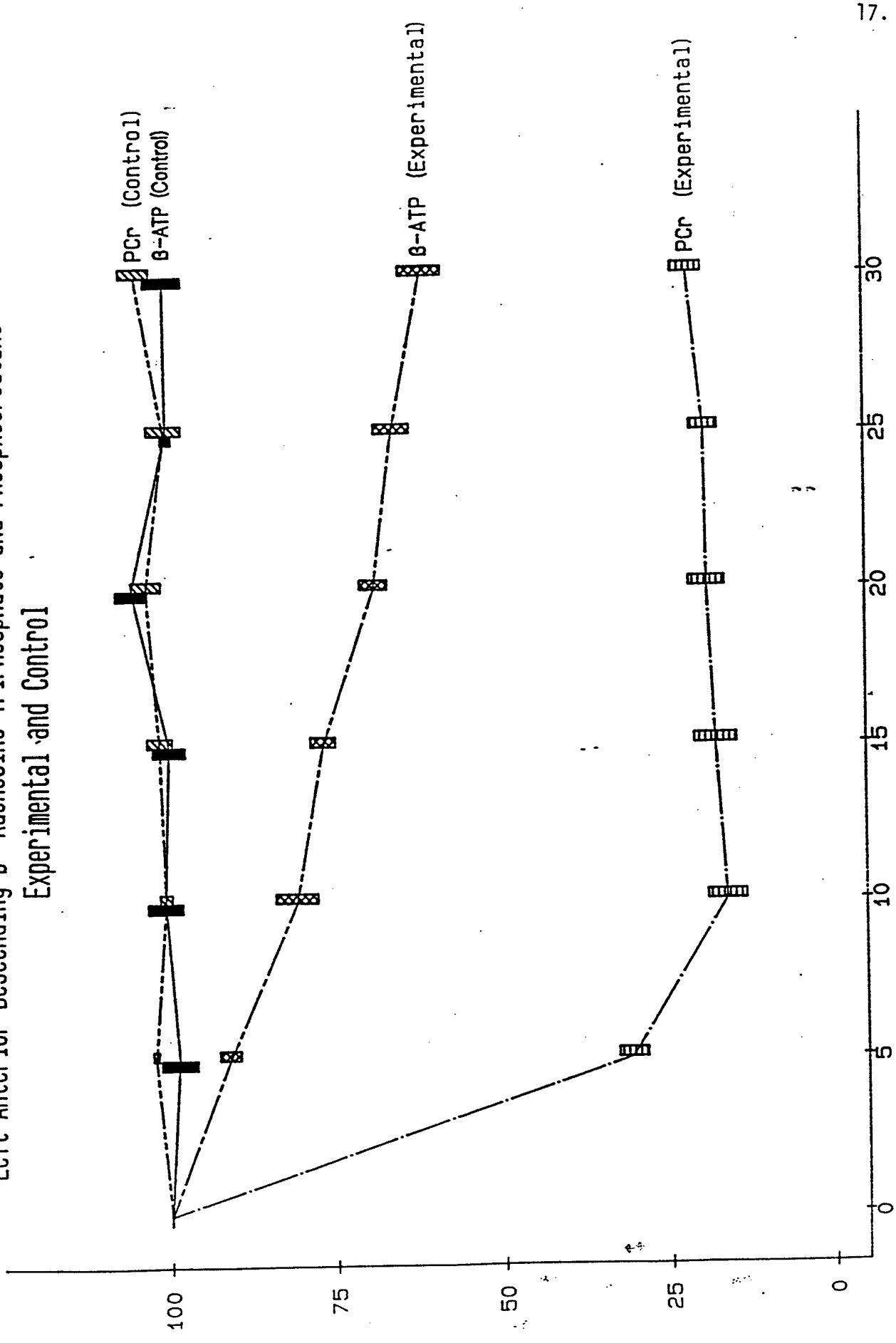

FIGURE 5: MYOCARDIAL METABOLITE CHANGES DURING THIRTY MINUTES OF ISCHEMIA

TABLE I: HEMODYNAMIC AND PH DATA

RESULTS

BASELINE

	CONTROL (n = 7)		EXPERIMENTAL (n = 7)
MAP	102 ± 5		108 ± 5
LVEDP	7 ± 1	*	12 ± 2
Heart Rate	114 ± 8		128 ± 9
+ dp/dt max	936 ± 48	*	1200 ± 59
% Systolic Shortening LAD	11.9 ± 1.1		11.3 ± 1.2
% Systolic Shortening LCX	7.8 ± 1.7		9.6 ± 1.3
Arterial pH	7.36 ± 0.02		7.37 ± 0.01
Extravascular Myocardial pH	7.35 ± 0.04		7.38 ± 0.02
NMR Myocardial pH	7.20 ± 0.01		7.19 ± 0.02

MEAN ± SEM
MAP = Mean Arterial Pressure (mmHg)
LVEDP = Left Ventricular End Diastolic Pressure (mmHg)
+ dp/dt max = Maximum Positive Rate of Left Ventricular Pressure Development (mmHg/sec)
LAD = Left Anterior Descending
LCX = Left Circumflex
* ANOVA $p \leq 0.05$

TABLE II: LINEAR REGRESSION OF MYOCARDIAL PH

RESULTS

LINEAR REGRESSION

NMR Derived Myocardial pH vs Extravascular Myocardial pH During Ischemia

ISCHEMIC DOG	*r*	*p*
1	0.96	0.0001
2	0.99	0.0001
3	0.96	0.0001
4	0.97	0.0001
5	0.96	0.0001
6	0.99	0.0001
7	0.88	0.0001
MEAN	0.96	

TABLE III: LINEAR REGRESSION OF HIGH ENERGY PHOSPHATES

RESULTS

LINEAR REGRESSION

Extravascular Myocardial pH vs % Control ATP During Ischemia

ISCHEMIC DOG	*r*	*p*
1	0.84	0.0003
2	0.95	0.0001
3	0.95	0.0001
4	0.95	0.0001
5	0.98	0.0001
6	0.96	0.0001
7	0.92	0.0001
MEAN	0.94	